FILE 'REGISTRY' ENTERED AT 10:27:54 ON 16 OCT 2003

=> S N-ACETYGLUCOSAMINE-1-PHOSPHATE/CN
L1 0 N-ACETYGLUCOSAMINE-1-PHOSPHATE/CN

=> S N-ACETYLGLUCOSAMINE-1-PHOSPHATE/CN
L2 1 N-ACETYLGLUCOSAMINE-1-PHOSPHATE/CN

=> D

L2 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2003 ACS on STN
RN 28446-21-1 REGISTRY
CN .alpha.-D-Glucopyranose, 2-(acetylamino)-2-deoxy-, 1-(dihydrogen phosphate) (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN .alpha.-D-Glucopyranose, 2-acetamido-2-deoxy-, 1-phosphate (7CI)
CN .alpha.-D-Glucosamine, N-acetyl-, 1-phosphate (6CI)
CN Glucopyranose, 2-acetamido-2-deoxy-, 1-(dihydrogen phosphate), .alpha.-D- (8CI)
OTHER NAMES:
CN .alpha.-D-N-Acetylglucosamine 1-phosphate
CN .alpha.-N-Acetylglucosamine 1-phosphate
CN N-Acetylglucosamine .alpha.-1-phosphate
CN N-Acetylglucosamine 1-phosphate
CN ***N-Acetylglucosamine-1-phosphate***
FS STEREOSEARCH
DR 22332-11-2, 139883-91-3, 92541-49-6
MF C8 H16 N O9 P
CI COM
LC STN Files: BEILSTEIN*, BIOSIS, CA, CAOLD, CAPLUS, TOXCENTER, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry.

/ Structure 1 in file .gra /

PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

61 REFERENCES IN FILE CA (1907 TO DATE)
2 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
61 REFERENCES IN FILE CAPLUS (1907 TO DATE)
2 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

=> S ALPHA-GLCNACASE
2620016 ALPHA
12 ALPHAS
2620016 ALPHA
(ALPHA OR ALPHAS)
2 GLCNACASE
L3 2 ALPHA-GLCNACASE
(ALPHA(W)GLCNACASE)

=> D 1-2

L3 ANSWER 1 OF 2 REGISTRY COPYRIGHT 2003 ACS on STN
RN 562882-93-3 REGISTRY
CN Phosphodiesterase, .alpha.-acetylglucosaminyloligosaccharide (human) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 18: PN: US20030133924 SEQID: 18 claimed protein
CN ***Phosphodiester .alpha.-GlcNAcase (human)***
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS, USPATFULL

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L3 ANSWER 2 OF 2 REGISTRY COPYRIGHT 2003 ACS on STN
RN 562882-90-0 REGISTRY
CN DNA (human .alpha.-acetylglucosaminyloligosaccharide phosphodiesterase cDNA plus 3'-flank) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 17: PN: US20030133924 SEQID: 17 claimed DNA
CN ***DNA (human phosphodiester .alpha.-GlcNAcase cDNA plus 3'-flank)***
FS NUCLEIC ACID SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS, USPATFULL

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

FILE 'CAPLUS' ENTERED AT 10:30:22 ON 16 OCT 2003

=> S N-ACETYLGLUCOSAMINE-1-PHOSPHATE
2631391 N
10850 ACETYLGLUCOSAMINE
49 ACETYLGLUCOSAMINES
10873 ACETYLGLUCOSAMINE
(ACETYLGLUCOSAMINE OR ACETYLGLUCOSAMINES)
7701266 1
490810 PHOSPHATE
119016 PHOSPHATES
535941 PHOSPHATE
(PHOSPHATE OR PHOSPHATES)
L4 185 N-ACETYLGLUCOSAMINE-1-PHOSPHATE
(N(W)ACETYLGLUCOSAMINE(W)1(W)PHOSPHATE)

=> S L4,L2
61 L2
L5 221 (L4 OR L2)

=> S ALPHA-GLCNACASE
1438918 ALPHA
2483 ALPHAS
1439012 ALPHA
(ALPHA OR ALPHAS)
59 GLCNACASE
4 GLCNACASES
60 GLCNACASE
(GLCNACASE OR GLCNACASES)
L6 8 ALPHA-GLCNACASE
(ALPHA(W)GLCNACASE)

=> S L6,L3
1 L3
L7 8 (L6 OR L3)

=> S ACETYLGLUCOSAMINYLOLIGOSACCHARIDE (2W) PHOSPHODIESTERASE
1 ACETYLGLUCOSAMINYLOLIGOSACCHARIDE
22450 PHOSPHODIESTERASE
2393 PHOSPHODIESTERASES
22917 PHOSPHODIESTERASE
(PHOSPHODIESTERASE OR PHOSPHODIESTERASES)
L8 1 ACETYLGLUCOSAMINYLOLIGOSACCHARIDE (2W) PHOSPHODIESTERASE

=> E ACETYLGLUCOSAMINYLOLIGOSACCHARIDE (2W) PHOSPHODIESTERASE
=> S E2
L9 1 ACETYLGLUCOSAMINYLOLIGOSACCHARIDE/BI

=> S E2(3W)PHOSPHODIESTERASE
1 ACETYLGLUCOSAMINYLOLIGOSACCHARIDE/BI
22450 PHOSPHODIESTERASE
2393 PHOSPHODIESTERASES
22917 PHOSPHODIESTERASE
(PHOSPHODIESTERASE OR PHOSPHODIESTERASES)
L10 1 ACETYLGLUCOSAMINYLOLIGOSACCHARIDE/BI(3W)PHOSPHODIESTERASE

=> D CBIB ABS

L10 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2003 ACS on STN
1997:151992 Document No. 126:182923 N-Acetylglucosamine-1-phosphodiester-.alpha.-N-acetylglucosaminidase. Mullis, Karen G. (Division of Hematology/Oncology, Washington Univ. Sch. of Medicine, St. Louis, MO, 63110-1093, USA). Guidebook to the Secretory Pathway, 278-279. Editor(s): Rothblatt, Jonathan; Novick, Peter; Stevens, Tom H. Oxford University Press: Oxford, UK. (English) 1994. CODEN: 64AJAT.
AB A review with 6 refs. N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase (uncovering enzyme; .alpha.-***acetylglucosaminyloligosaccharide*** ***phosphodiesterase*** ; EC 3.1.4.45) is a membrane-bound Golgi enzyme that removes the N-acetylglucosamine residue from N-acetylglucosamine-phosphate-mannose (GlcNAc-P-Man) groups on oligosaccharides of newly synthesized lysosomal enzymes. The resulting mannose 6-phosphate (Man 6-P) serves as the recognition marker for binding Man 6-P receptors and transport to the lysosomes.

=> S UNCOVERING ENZYME?
998 UNCOVERING
2 UNCOVERINGS
1000 UNCOVERING
(UNCOVERING OR UNCOVERINGS)
864679 ENZYME?
L11 9 UNCOVERING ENZYME?
(UNCOVERING(W)ENZYME?)

=> S L11 NOT L10
L12 8 L11 NOT L10

=> D 1-8 CBIB ABS

L12 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
2002:646182 Document No. 138:182906 Human mannose 6-phosphate-***uncovering*** ***enzyme*** is synthesized as a proenzyme that is activated by the endoprotease furin. Do, Hung; Lee, Wang-Sik; Ghosh, Pradipta; Hollowell, Tracy; Canfield, William; Kornfeld, Stuart (Novazyme Pharmaceuticals, Incorporated, Oklahoma City, OK, 73104, USA). Journal of Biological Chemistry, 277(33), 29737-29744 (English) 2002. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB N-Acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase, also known as " ***uncovering*** " ***enzyme*** (UCE), is localized in the trans-Golgi network, where it removes a covering N-acetylglucosamine from the mannose 6-phosphate recognition market on lysosomal acid hydrolases. Here we show that UCE is synthesized as an inactive proenzyme that is activated by the endoprotease furin, which cleaves an RARLPR .dwnarw. D sequence to release a 24-amino acid propiece. As furin is localized in the trans-Golgi network, newly synthesized UCE is inactive until it reaches this terminal Golgi compartment. LoVo cells (derived from a human colon adenocarcinoma) lack furin activity and have extremely low UCE activity. Addn. of furin to LoVo cell exts. restores UCE activity to normal levels, demonstrating that the UCE proenzyme is stable in this cell type. LoVo cells secrete acid hydrolases with phosphomannose diesters as a consequence of the deficient UCE activity. This demonstrates for the first time that UCE is the only enzyme in these cells capable of efficiently uncovering phosphomannose diesters. UCE also

Hydrolyzes UDP-GlcNAc, a sugar donor for Golgi N-acetylglucosaminyl-transferases. The fact that UCE is not activated until it reaches the trans-Golgi network may ensure that the pool of UDP-GlcNAc in the Golgi stack is not depleted, thereby maintaining proper oligosaccharide assembly.

L12 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
2002:102761 Document No. 136:243730 Multiple signals regulate trafficking of the mannose 6-phosphate- ***uncovering*** ***enzyme*** . Lee, Wang-Sik; Rohrer, Jack; Kornfeld, Rosalind; Kornfeld, Stuart (Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, 63110, USA). Journal of Biological Chemistry, 277(5), 3544-3551 (English) 2002. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB The " ***uncovering*** ***enzyme*** ," which catalyzes the second step in the formation of the mannose 6-phosphate recognition marker on lysosomal enzyme oligosaccharides, resides primarily in the trans-Golgi network and cycles between this compartment and the plasma membrane. An anal. of green fluorescent protein- ***uncovering*** ***enzyme*** chimeras revealed that the transmembrane segment and the first 11 residues of the 41-residue-cytoplasmic tail are sufficient for retention in the trans-Golgi network. The next eight residues (486YAYHPLQE493) facilitate exit from this compartment. Kinetic studies demonstrated that the 488YHPL491 sequence also mediates rapid internalization at the plasma membrane. This motif binds adaptor protein-2 in glutathione S-transferase- ***uncovering*** ***enzyme*** -cytoplasmic tail pull-down assays, indicating that the ***uncovering*** ***enzyme*** is endocytosed via clathrin-coated vesicles. Consistent with this finding, endogenous ***uncovering*** ***enzyme*** was detected in purified clathrin-coated vesicles. The enzyme with a Y486A mutation is internalized normally but accumulates on the cell surface because of increased recycling to the plasma membrane. This residue is required for efficient return of the enzyme from endosomes to the trans-Golgi network. These findings indicate that the YAYHPLQE motif is recognized at several sorting sites, including the trans-Golgi network, the plasma membrane, and the endosome.

L12 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
2001:800029 Document No. 136:50263 Lysosomal hydrolase mannose 6-phosphate ***uncovering*** ***enzyme*** resides in the trans-Golgi network. Rohrer, Jack; Kornfeld, Rosalind (Friedrich Miescher Institut, Basel, 4059, Switz.). Molecular Biology of the Cell, 12(6), 1623-1631 (English) 2001. CODEN: MBCEEV. ISSN: 1059-1524. Publisher: American Society for Cell Biology.
AB A crucial step in lysosomal biogenesis is catalyzed by " ***uncovering*** " ***enzyme*** (UCE), which removes a covering N-acetylglucosamine from the mannose 6-phosphate (Man-6-P) recognition marker on lysosomal hydrolases. This study shows that UCE resides in the trans-Golgi network (TGN) and cycles between the TGN and plasma membrane. The cytosolic domain of UCE contains two potential endocytosis motifs: 488YHPL and C-terminal 511NPFKD. YHPL is shown to be the more potent of the two in retrieval of UCE from the plasma membrane. A green-fluorescent protein-UCE transmembrane-cytosolic domain fusion protein colocalizes with TGN 46, as does endogenous UCE in HeLa cells, showing that the transmembrane and cytosolic domains det. intracellular location. These data imply that the Man-6-P recognition marker is formed in the TGN, the compartment where Man-6-P receptors bind cargo and are packaged into clathrin-coated vesicles.

L12 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
2001:543361 Document No. 136:198139 Biochemical analysis of mutations in palmitoyl-protein thioesterase causing infantile and late-onset forms of neuronal ceroid lipofuscinosis. Das, Amit K.; Lu, Jui-Yun; Hofmann, Sandra L. (Department of Internal Medicine and the Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas, TX, 75390-8593, USA). Human Molecular Genetics, 10(13), 1431-1439 (English) 2001. CODEN: HMGEE5. ISSN: 0964-6906. Publisher: Oxford University Press.
AB Deficiency in a recently characterized lysosomal enzyme, palmitoyl-protein thioesterase (PPT), leads to a severe neurodegenerative disorder of children, infantile neuronal ceroid lipofuscinosis (NCL). Over 36

different mutations in the PPT gene have been described, and missense mutations have been interpreted in the light of the recently solved x-ray crystallog. structure of PPT. In the current study, the authors assessed the biochem. impact of mutations through the study of cells derived from patients and from the expression of recombinant PPT enzymes in COS and Sf9 cells. All missense mutations assocd. with infantile NCL showed no residual enzyme activity, whereas mutations assocd. with late-onset phenotypes showed up to 2.15% residual activity. Two mutations increased the Km of the enzyme for palmitoylated substrates and were located in positions that would distort the palmitate-binding pocket. An initiator methionine mutation (ATG.fwdarw.ATA) in two late-onset patients was expressed at a significant level in COS cells, suggesting that the ATA codon may be utilized to a clin. important extent in vivo. The most common PPT nonsense mutation, R151X, was assocd. with an absence of PPT mRNA. Mannose 6-phosphate modification of wild-type and mutant PPT enzymes was grossly normal at the level of the phosphotransferase reaction. However, mutant PPT enzymes did not bind to mannose 6-phosphate receptors in a blotting assay. This observation was related to the failure of the mutant expressed enzymes to gain access to " ***uncovering*** ***enzyme*** " (N-acetylglucosamine-1-phosphodiester .alpha.-N-acetyl glucosaminidase), presumably due to a block in transit out of the endoplasmic reticulum, where mutant enzymes are degraded.

L12 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN

1996:666410 Document No. 125:321280 Purification and characterization of human lymphoblast N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase. Page, Theodore; Zhao, Ke-Wei; Tao, Ling; Miller, Arnold L. (Department of Neurosciences 0624, University of California, San Diego, La Jolla, CA, 92093-0624, USA). Glycobiology, 6(6), 619-626 (English) 1996. CODEN: GLYCE3. ISSN: 0959-6658. Publisher: Oxford University Press.

AB N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase (EC 3.1.4.45; ***uncovering*** ***enzyme***) (I) catalyzes the removal of N-acetylglucosamine from the N-acetylglucosamine-.alpha.-phospho-mannose portion of selected lysosomal enzyme oligosaccharide chains, thereby forming the mannose 6-phosphate signal which is responsible for the targeting of these lysosomal enzymes for transport into lysosomes. I was purified .apprx.7000-fold to electrophoretic homogeneity from Epstein-Barr virus-transformed human lymphoblast cells. The purifn. sequence involved solubilizing this membrane-bound enzyme with Tergitol NP-10, affinity chromatog. on lentil lectin-Sepharose 4B, ion-exchange chromatog. on DEAE-Sephacel, chromatog. on Zn(II)-IDA-Sepharose 6B, and preparative SDS-PAGE electrophoresis. Purified I migrated as a single band of 114 kDa which was coincident with enzyme activity on anal. SDS-PAGE electrophoresis. Characterization studies of purified I demonstrated that catalytic activity was maximal at pH 6.95 and that the enzyme retained full activity following incubation for 10 min at 60.degree.. No requirement was found for a divalent cation, but Zn2+, Hg2+, and Cu2+ were found to reduce I activity by 30-40%. The highest catalytic efficiency was obsd. with N-acetylglucosamine-phospho-methylmannoside as a substrate, whereas UDP-N-acetylglucosamine, N-acetylglucosamine-phosphomannose-uteroferrin, and N-acetylglucosamine phosphate were also cleaved by I with decreasing efficiency. 6-Acetamido-6-deoxycastanospermine was a potent inhibitor of human I with a Ki of 0.35 .mu.M, whereas N-acetylglucosamine phosphate (Ki = 1.58 mM) and N-acetylglucosamine (Ki = 5.1 mM) inhibited I to a lesser degree.

L12 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN

1993:163736 Document No. 118:163736 The synthesis of substrates and two assays for the detection of N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase (***uncovering*** ***enzyme***). Mullis, Karen Gheesling; Ketcham, Catherine M. (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Analytical Biochemistry, 205(2), 200-7 (English) 1992. CODEN: ANBCA2. ISSN: 0003-2697.

AB A method for the synthesis and purifn. of large quantities of 4 radiolabeled substrates for quantitation of N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase (***uncovering*** ***enzyme***) (I) is described. Four substrates, [3H]GlcNAc-.alpha.-P-Man.alpha.Me, [3H]GlcNAc-.alpha.-P-uteroferrin, [3H]GlcNAc.alpha.-P-Man.alpha.1-2Man-O-Me, and [3H]GlcNAc.alpha.-P-Man9GlcNAc, were enzymically synthesized using acetylglucosaminephosphotransferase from

Acanthamoeba castellanii and UDP-N-acetyl-[3H]glucosamine and, as acceptor, methyl-.alpha.-D-mannopyranoside (Man.alpha.Me), uteroferrin, Man.alpha.1-2Man-O-Me, or Man9GlcNAc. The isolation of the [3H]GlcNAc-P-modified product of each reaction is detailed. Two assays for the detection of I activity using [3H]GlcNAc-.alpha.-P-uteroferrin and [3H]GlcNAc-.alpha.-P-Man.alpha.Me are outlined. The ability to easily synthesize 4 relevant substrates for I offers flexibility in assaying I.

L12 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
1990:547654 Document No. 113:147654 Suppression of the 'uncovering' of mannose-6-phosphate residues in lysosomal enzymes in the presence of ammonium chloride. Isidoro, Ciro; Radons, Juergen; Baccino, Francesco M.; Hasilik, Andrej (Inst. Physiol. Chem. Pathobiochem., Westfael. Wilhelms-Univ., Muenster, D-4400, Germany). European Journal of Biochemistry, 191(3), 591-7 (English) 1990. CODEN: EJBCAI. ISSN: 0014-2956.
AB The uncovering ratio of phosphate groups in lysosomal enzymes is defined as the percentage of phosphomonoester groups in the oligosaccharide side chains based on the sum of phosphomonoester and phosphodiester groups. Using a new procedure for the specific and complete hydrolysis of uncovered phosphomonoester groups in denatured immunoppts. of human cathepsin D. It is shown that the uncovering ratio varies between different forms of the enzyme and may be used as an indicator of the maturation of its carbohydrate side chains. The uncovering ratio in the total (cellular and secreted) cathepsin D from U937 promonocytes is >95%. It is only slightly decreased in cells incubated in the presence of 1.alpha.,25-dihydroxycholecalciferol, in which the rate of synthesis of cathepsin D is several-times-higher than in the control cells. In U937 cells and also in fibroblasts, the uncovering is nearly complete in mature forms of the intracellular cathepsin D but less extensive in the intracellular and secreted precursor. In both cell types, incubation with 10 mM NH4Cl results in a decrease in the uncovering ratio of total cathepsin D. However, the activity of the ***uncovering*** ***enzyme*** , N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase, as detd. with UDP-N-acetylglucosamine is not affected with up to 60 mM NH4Cl. These results suggest that NH4Cl, in addn. to its known effects on the acidic-pH-dependent functions of lysosomal compartments and of mannose-6-phosphate receptors, impairs the processing or transport of lysosomal enzyme precursors at, or proximally to, the site of the uncovering of their mannose-6-phosphate residues.

L12 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
1990:526117 Document No. 113:126117 Brefeldin A prevents uncovering but not phosphorylation of the recognition marker in cathepsin D. Radons, Juergen; Isidoro, Ciro; Hasilik, Andrej (Inst. Physiol. Chem. Pathobiochem., Westfaelische Wilhelms-Univ., Muenster, D-4400, Germany). Biological Chemistry Hoppe-Seyler, 371(7), 567-73 (English) 1990. CODEN: BCHSEI. ISSN: 0177-3593.
AB Brefeldin A (BFA) has been shown to inhibit transiently the subcellular transport of cathepsin D. The effect of this antibiotic on processing of the phosphorylated oligosaccharides in cathepsin D was studied in human promonocytes U937. In the presence of the drug the phosphorylation of cathepsin D precursor continued at a diminished rate. The phosphorylated oligosaccharides in cathepsin D comprised mono- and bis-phosphorylated forms. The relative amts. of the two species were not changed in the presence of BFA. The uncovering of the phosphate groups and the proteolytic processing of the phosphorylated precursor were abolished. In an in vitro assay the ***uncovering*** ***enzyme*** , N-acetylglucosamine-1-phosphodiester N-acetylglucosaminidase was not inhibited by BFA. Thus, this drug interrupts the traffic between the compartments contg. N-acetylglucosaminyl phosphotransferase and N-acetylglucosamine-1-phosphodiester N-acetylglucosaminidase.

=> S PSEUDOMONAS;S EXOTOXIN;S EXODOTOXIN
65082 PSEUDOMONAS
18 PSEUDOMONADES
L13 65086 PSEUDOMONAS
(PSEUDOMONAS OR PSEUDOMONADES)

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          2937 EXOTOXIN
          1099 EXOTOXINS
L14       3374 EXOTOXIN
                 (EXOTOXIN OR EXOTOXINS)


             0 EXODOTOXIN
L15          0 EXODOTOXIN

=> S L14(W)A
      17406370 A
L16       1123 L14(W)A

=> S L13 AND L16
L17        913 L13 AND L16

=> S L13(6A)L16
L18        814 L13(6A)L16

=> S L4 AND L18
L19          2 L4 AND L18

=> S L19 NOT (L9,L12)
L20          2 L19 NOT ((L9 OR L12))

=> D 1-2 CBIB ABS

L20  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2003 ACS on STN
2003:648627   Correction of: 2003:551314   Document No. 139:161504   Correction
     of: 139:113661  Expression of .alpha.-N-acetylglucosaminyl
     phosphodiesterase proenzyme in furin-deficient mammalian cells and its use
     in production of lysosomal hydrolases with modified oligosaccharide moiety
     for treatment of lysosomal storage disease.  Canfield, William M.;
     Kornfeld, Stuart (Genzyme Glycobiology Research Institute, Inc., USA).
     PCT Int. Appl. WO 2003057138 A2 20030717, 31 pp.  DESIGNATED STATES: W:
     AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR,
     CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
     MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG,
     SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM,
     AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM,
     CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT,
     SE, SN, TD, TG, TR.  (English).  CODEN: PIXXD2.  APPLICATION: WO
     2002-US38976 20021220.  PRIORITY: US 2001-23894 20011221.
AB   The present invention provides methods of producing a pro-N-
     acetylglucosamine-1-phosphodiester .alpha.-N-acetyl glucosaminidase
     (phosphodiester .alpha.-GlcNAcase; .alpha.-N-acetylglucosaminyl
     phosphodiesterase), in mammalian cells deficient in the furin proteolytic
     enzyme and methods of making lysosomal hydrolases having an   ***N***  -
       ***acetylglucosamine***  -  ***1***  -  ***phosphate***  .  The
     phosphodiester .alpha.-GlcNAcase comprises a pro-peptide sequence between
     the signal sequence and the sequence of the mature active form.  This
     pro-peptide sequence is proteolytically cleaved to yield the mature active
     form of phosphodiester .alpha.-GlcNAcase.  The activity of uncleaved
     phosphodiester .alpha.-GlcNAcase was significantly lower than the activity
     of the phosphodiester .alpha.-GlcNAcase when the pro-peptide sequence was
     cleaved.  The pro-peptide sequence contains a recognition site for the
     site-specific protease furin which mediates cleavage of phosphodiester
     .alpha.-GlcNAcase to its mature form.  Based on this finding, the
     invention provides processes of making lysosomal hydrolase in cells which
     are deficient in furin and thus have the uncleaved form of phosphodiester
     .alpha.-GlcNAcase.  By making the lysosomal hydrolases in these cells, the
     lysosomal hydrolase is modified with an   ***N***  -
       ***acetylglucosamine***  -  ***1***  -  ***phosphate***   moiety and is
     not removed, or removed at a low efficiency.  After expression and
     recovery of the lysosomal hydrolase from these furin deficient cells, the
     lysosomal hydrolase can be treated with an active form of phosphodiester
     .alpha.-GlcNAcase thereby removing the N-acetylglucosamine moiety to yield
     a highly phosphorylated lysosomal enzyme, which can be used in enzyme
     replacement therapies to treat patients suffering from lysosomal storage
     diseases.  Thus, the method facilitates a simple and reliable method of
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producing lysosomal hydrolases with the appropriate phospho-modifications thereby reducing the steps necessary to produce a lysosomal hydrolase for therapeutic or exptl. use. Another object of the present invention is methods for producing a phosphodiester .alpha.-GlcNAcase having its pro-peptide intact by culturing cells or selecting cells that are furin deficient, where the selection is preferably conducted using ***Pseudomonas*** ***exotoxin*** ***A*** .

L20 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN
2003:551314 Document No. 139:113661 Expression of .alpha.-N-acetylglucosaminyl phosphodiesterase proenzyme in furin-deficient mammalian cells and its use in production of lysosomal hydrolases with modified oligosaccharide moiety for treatment of lysosomal storage disease. Canfield, William M.; Kornfeld, Stuart (Genzyme Glycobiology Research Institute, Inc., USA). PCT Int. Appl. WO 2003057138 A2 20030717, 31 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US38976 20021220. PRIORITY: US 2001-23894 20011221.

AB The present invention provides methods of producing a pro-N-acetylglucosamine-1-phosphodiester .alpha.-N-acetyl glucosaminidase (phosphodiester .alpha.-GlcNAcase; .alpha.-N-acetylglucosaminyl phosphodiesterase), in mammalian cells deficient in the furin proteolytic enzyme and methods of making lysosomal hydrolases having an ***N*** - ***acetylglucosamine*** - ***1*** - ***phosphate*** . The phosphodiester .alpha.-GlcNAcase comprises a pro-peptide sequence between the signal sequence and the sequence of the mature active form. This pro-peptide sequence is proteolytically cleaved to yield the mature active form of phosphodiester .alpha.-GlcNAcase. The activity of uncleaved phosphodiester .alpha.-GlcNAcase was significantly lower than the activity of the phosphodiester .alpha.-GlcNAcase when the pro-peptide sequence was cleaved. The pro-peptide sequence contains a recognition site for the site-specific protease furin which mediates cleavage of phosphodiester .alpha.-GlcNAcase to its mature form. Based on this finding, the invention provides processes of making lysosomal hydrolase in cells which are deficient in furin and thus have the uncleaved form of phosphodiester .alpha.-GlcNAcase. By making the lysosomal hydrolases in these cells, the lysosomal hydrolase is modified with an ***N*** - ***acetylglucosamine*** - ***1*** - ***phosphate*** moiety and is not removed, or removed at a low efficiency. After expression and recovery of the lysosomal hydrolase from these furin deficient cells, the lysosomal hydrolase can be treated with an active form of phosphodiester .alpha.-GlcNAcase thereby removing the N-acetylglucosamine moiety to yield a highly phosphorylated lysosomal enzyme, which can be used in enzyme replacement therapies to treat patients suffering from lysosomal storage diseases. Thus, the method facilitates a simple and reliable method of producing lysosomal hydrolases with the appropriate phospho-modifications thereby reducing the steps necessary to produce a lysosomal hydrolase for therapeutic or exptl. use. Another object of the present invention is methods for producing a phosphodiester .alpha.-GlcNAcase having its pro-peptide intact by culturing cells or selecting cells that are furin deficient, where the selection is preferably conducted using ***Pseudomonas*** ***exotoxin*** ***A*** .

=> S LYSOSOM?;S HYDROLASE;S L21(3A)L22
L21 33020 LYSOSOM?

17526 HYDROLASE
7769 HYDROLASES
L22 21577 HYDROLASE
(HYDROLASE OR HYDROLASES)

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L23          1790 L21(3A)L22

=> S L23 AND L18
L24             2 L23 AND L18

=> S L23 AND L17
L25             2 L23 AND L17

=> S L23 AND L16
L26             2 L23 AND L16

=> S (L24,L25,L26) NOT L20
L27             0 ((L24 OR L25 OR L26)) NOT L20

=> E CANFIELD W/AU
=> S E4-E12
                1 "CANFIELD W B"/AU
                2 "CANFIELD W K"/AU
                1 "CANFIELD WESLEY"/AU
               13 "CANFIELD WESLEY K"/AU
                5 "CANFIELD WILLIAM"/AU
               24 "CANFIELD WILLIAM M"/AU
                1 "CANFIELD WILLIAM MONROE"/AU
               19 "CANFIELD WM B"/AU
                1 "CANFIELD WM R"/AU
L28            67 ("CANFIELD W B"/AU OR "CANFIELD W K"/AU OR "CANFIELD WESLEY"/AU
                  OR "CANFIELD WESLEY K"/AU OR "CANFIELD WILLIAM"/AU OR "CANFIELD
                  WILLIAM M"/AU OR "CANFIELD WILLIAM MONROE"/AU OR "CANFIELD WM
                  B"/AU OR "CANFIELD WM R"/AU)

=> E KORNFELD S/AU
=> S E3,E10-E12
                5 "KORNFELD S"/AU
              213 "KORNFELD STUART"/AU
                1 "KORNFELD STUART A"/AU
                1 "KORNFELD STURAT"/AU
L29           220 ("KORNFELD S"/AU OR "KORNFELD STUART"/AU OR "KORNFELD STUART
                  A"/AU OR "KORNFELD STURAT"/AU)

=> S L28,L29
L30           281 (L28 OR L29)

=> S L30 AND L4
L31            10 L30 AND L4

=> S L30 AND L5
L32            12 L30 AND L5

=> S L30 AND L16
L33             2 L30 AND L16

=> S (L32,L33) NOT (L20,L12,L10)
L34            10 ((L32 OR L33)) NOT ((L20 OR L12 OR L10))

=> D 1-10 CBIB ABS

L34  ANSWER 1 OF 10  CAPLUS  COPYRIGHT 2003 ACS on STN
2003:550993   Document No. 139:112730  Method for production of highly
     phosphorylated human acid .beta.-glucocerebrosidase (GBA), and use of GBA
     in treating bone or lung tissue of patient with Gaucher's disease.
       ***Canfield, William***   (Novazyme Pharmaceuticals, Inc., USA).  U.S.
     Pat. Appl. Publ. US 2003133924 A1 20030717, 54 pp.  (English).  CODEN:
     USXXCO.  APPLICATION: US 2001-24197 20011221.
AB   The invention provides a method for producing a highly phosphorylated acid
     .beta.-glucocerebrosidase (GBA), which involves: (a) culturing cells
     transfected with polynucleotides encoding a recombinant GBA in the
     presence of at least one .alpha.1,2-mannosidase inhibitor; (b) recovering
     high mannose recombinant GBA from said cells; (c) contacting said GBA with
     an isolated N-acetylglucosaminyl phosphotransferase (GlcNAc
     phosphotransferase) to produce a modified GBA; and (d) contacting said
     modified GBA with N-acetylglucosamine-1-phosphodiester
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.alpha.-N-acetylglucosaminidase (phosphodiester .alpha.-GlcNAcase). The invention also provides for the use of said highly phosphorylated GBA in treating bone or lung tissue of a patient suffering from Gaucher's disease. The invention further provides the cDNA and amino acid sequences of human GBA, phosphodiester .alpha.-GlcNAcase, and GlcNAc phosphotransferase. The invention relates that said GlcNAc phosphotransferase comprises an .alpha. and .beta. subunit, which reduces substrate specificity, and allows the GlcNAc phosphotransferase to catalyze the transfer of ***N*** - ***acetylglucosamine*** - ***1*** - ***phosphate*** from UDP-GlcNAc to the GBA enzyme. The invention discussed that in this method GBA will be phosphorylated which will allow binding to mannose 6 receptors on the surface of lung and bone cells. In so binding to the receptor on these tissues, the problems of the current GBA replacement therapy can be addressed.

L34 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

2003:492554 Document No. 139:65404 Soluble human acetylglucosamine-1-phosphotransferase containing an artificial proteolytic cleavage site to generate .alpha. and .beta. subunits. ***Canfield, William*** ; Kudo, Mariko (Novazyme Pharmaceuticals, Inc., USA). U.S. Pat. Appl. Publ. US 2003119088 A1 20030626, 55 pp. (English). CODEN: USXXCO. APPLICATION: US 2001-23888 20011221.

AB Recombinant sol. UDP-N-acetylglucosamine:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase (I, EC 2.7.8.17) is not efficiently subject to post-translational proteolytic cleavage when expressed in mammalian cells and uncleaved forms have poor GlcNAc phosphotransferase activity. To solve this problem, the invention shows that by interposing a unique proteolytic cleavage site between the .alpha. and .beta. subunits in the I polyprotein, the polyprotein is cleaved and when expressed with the .gamma. subunit, effectively phosphorylates an enzyme substrate. In addn., the .alpha. and .beta. subunits alone without the .gamma. subunit are catalytically active. Furthermore, the absence of the .gamma. subunit results in loss of substrate specificity to only those lysosomal enzymes targeted via the mannose-6-phosphate targeting systems, e.g., acid .alpha.-glucosidase, acid .beta.-galactosidase, .beta.-hexaminidase, and others. This loss of substrate specificity allows the sol. I contg. the .alpha. and .beta. tetramer to effectively phosphorylate any glycoprotein having an appropriate acceptor oligosaccharide. Patients suffering from a lysosomal storage disease can be treated by contacting a lysosomal hydrolase with sol. I to produce a lysosomal hydrolase with an ***N*** - ***acetylglucosamine*** - ***1*** - ***phosphate*** , removing the N-acetylglucosamine by contact of the lysosomal hydrolase with a N-acetylglucosamine-1-phosphodiester-N-acetylglucosaminidase (EC 3.1.4.45) to produce a phosphorylated lysosomal hydrolase, and administering an amt. of the phosphorylated enzyme sufficient to treat said disease.

L34 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

2001:208390 Document No. 134:248843 Use of GlcNAc-phosphotransferase and phosphodiester .alpha.-GlcNAcase in production of highly phosphorylated lysosomal hydrolases useful in treatment of lysosomal storage diseases. ***Canfield, William M.*** (USA). PCT Int. Appl. WO 2001019955 A2 20010322, 91 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US21970 20000914. PRIORITY: US 1999-PV153831 19990914.

AB The lysosomal targeting pathway enzymes GlcNAc-phosphotransferase and phosphodiester .alpha.-GlcNAcase and uses in prodn. of highly phosphorylated lysosomal hydrolases that can be used to treat lysosomal storage diseases, are disclosed. Generally, the nucleic acid mols. coding for the enzymes are incorporated into expression vectors that are used to transfect host cells that express the enzymes. The expressed enzymes are recovered using monoclonal antibodies capable of selectively binding to bovine GlcNAc-phosphotransferase and to bovine phosphodiester .alpha.-GlcNAcase. Lysosomal hydrolases having high mannose structures are treated with GlcNAc-phosphotransferase and phosphodiester

.alpha.-GlcNAcase resulting in the prodn. of asparagine-linked oligosaccharides that are highly modified with mannose 6-phosphate ("M6P"). The treated hydrolase binds to M6P receptors on the cell membrane and is transported into the cell and delivered to the lysosome where it can perform its normal or a desired function. The highly phosphorylated lysosomal hydrolases are readily taken into the cell and into the lysosome during enzyme replacement therapy procedures.

L34 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN
1996:762180 Document No. 126:100959 Bovine UDP-N-acetylglucosamine:lysosomal-enzyme N-acetylglucosamine-1-phosphotransferase. I. Purification and subunit structure. Bao, Ming; Booth, J. Leland; Elmendorf, B. Jean; ***Canfield, William M.*** (W. K. Warren Med. Res. Inst., Univ. Oklahoma Health Sci. Cent., Oklahoma City, OK, 73104, USA). Journal of Biological Chemistry, 271(49), 31437-31445 (English) 1996. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Lysosomal enzyme N-acetylglucosamine-1-phosphotransferase (I) catalyzes the initial step in the synthesis of the mannose 6-phosphate determinant required for efficient intracellular targeting of newly synthesized lysosomal hydrolases to the lysosome. I was partially purified .apprx.30,000-fold by chromatog. of solubilized membrane proteins from lactating bovine mammary glands on DEAE-Sepharose, Reactive Green 19-agarose, and Superose 6. Partially purified I was used to generate a panel of murine monoclonal antibodies. Anti-I monoclonal antibody PT18 was coupled to a solid support and used to immunopurify I .apprx.480,000-fold to apparent homogeneity with an overall yield of 29%. Purified I had a specific activity of 10-12 .mu.mol ***N*** - ***acetylglucosamine*** - ***1*** - ***phosphate*** transferred/h/mg protein using 100 mM .alpha.-methylmannoside as acceptor. The subunit structure of I was detd. using a combination of anal. gel filtration chromatog., SDS-PAGE, and N-terminal sequencing. The results indicated that bovine I is a 540 kDa complex composed of disulfide-linked homodimers of 166- and 51-kDa subunits and 2 identical, noncovalently assocd. 56-kDa subunits.

L34 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN
1983:175528 Document No. 98:175528 Demonstration of the enzymatic mechanisms of .alpha.-N-acetyl-D-glucosamine-1-phosphodiester N-acetylglucosaminidase (formerly called .alpha.-N-acetylglucosaminylphosphodiesterase) and lysosomal .alpha.-N-acetylglucosaminidase. Varki, Ajit; Sherman, William; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Archives of Biochemistry and Biophysics, 222(1), 145-9 (English) 1983. CODEN: ABBIA4. ISSN: 0003-9861.

AB An enzyme that is capable of removing the outer N-acetylglucosamine residues from phosphodiesters present on the high-mannose-type oligosaccharides of newly synthesized lysosomal enzymes has been called an .alpha.-N-acetylglucosaminylphosphodiesterase, based upon its substrate specificity and on inhibitor studies. The 18O enrichment method was used to demonstrate that the enzyme cleaves the C-O bond rather than the O-P bond, and therefore acts by a glycosidase type of mechanism. In addn., the enzyme has no significant activity toward .alpha.- ***N*** - ***acetylglucosamine*** ***1*** - ***phosphate*** , and therefore requires an underlying phosphodiester for activity. In accordance with the IUB recommendation for enzyme nomenclature, it is therefore suggested that the enzyme be renamed .alpha.-N-acetyl-D-glucosamine-1-phosphodiester N-acetylglucosaminidase (systematic name, 2-acetamido-2-deoxy-.alpha.-D-glucose 1-phosphodiester acetamidodeoxyglucohydrolase). For convenience, the trivial name phosphodiester glycosidase is proposed. Lysosomal .alpha.-N-acetylglucosaminidase also has a glycosidase type of mechanism, but it is active toward .alpha.- ***N*** - ***acetylglucosamine*** ***1*** - ***phosphate*** as well as phosphodiesters with outer N-acetylglucosamine residues.

L34 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN
1983:69599 Document No. 98:69599 Lysosomal enzyme oligosaccharide phosphorylation in mouse lymphoma cells: specificity and kinetics of binding to the mannose 6-phosphate receptor in vivo. Gabel, Christopher A.; Goldberg, Daniel E.; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Journal of Cell Biology, 95(2 Pt. 1), 536-42 (English) 1982. CODEN: JCLBA3. ISSN: 0021-9525.

AB Phosphomannosyl residues on lysosomal enzymes serve as an essential component of the recognition marker necessary for binding to the mannose 6-phosphate (Man 6-P) receptor and translocation to lysosomes. The high mannose-type oligosaccharide units of lysosomal enzymes are phosphorylated by the following mechanism: ***N*** - ***acetylglucosamine*** ***1*** - ***phosphate*** is transferred to the 6 position of a mannose residue to form a phosphodiester; then the N-acetylglucosamine is removed to expose a phosphomonoester. The kinetics of this phosphorylation pathway were examd. in the murine lymphoma BW5147.3 cell line to det. the state of oligosaccharide phosphorylation at the time the newly synthesized lysosomal enzymes bind to the receptor. Cells were labeled with [2-3H]mannose for 20 min and then chased for various times .ltoreq.4 h. The binding of newly synthesized glycoproteins to the Man 6-P receptor was followed by eluting the bound ligand with Man 6-P. Receptor-bound material was first detected at 30 min of chase and reached a max. at 60 min of chase, at which time .apprx.10% of the total phosphorylated oligosaccharides were assocd. with the receptor. During longer chase times, the total quantity of cellular phosphorylated oligosaccharides decreased with a half-time of 1.4 h, suggesting that the lysosomal enzymes had reached their destination and had been dephosphorylated. The structures of the phosphorylated oligosaccharides of the eluted ligand were then detd. and compared with the phosphorylated oligosaccharides of mols. which were not bound to the receptor. The major phosphorylated oligosaccharide species present in the nonreceptor-bound material contained a single phosphodiester at all times examd. In contrast, receptor-bound oligosaccharides were greatly enriched in species possessing 1 and 2 phosphomonoesters. Evidently, binding of newly synthesized lysosomal enzymes to the Man 6-P receptor occurs only after removal of the covering N-acetylglucosamine residues.

L34 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

1982:101461 Document No. 96:101461 The phosphorylation of .beta.-glucuronidase oligosaccharides in mouse P388D1 cells. Goldberg, Daniel E.; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Journal of Biological Chemistry, 256(24), 13060-7 (English) 1981. CODEN: JBCHA3. ISSN: 0021-9258.

AB High-mannose-type oligosaccharides of acid hydrolases are phosphorylated by the transfer of ***N*** - ***acetylglucosamine*** ***1*** - ***phosphate*** to the 6 position of mannose. This is followed by removal of the covering N-acetylglucosamine residue to expose a phosphomonoester. The kinetics of this phosphorylation pathway were examd. in the murine macrophage line P388D1. Cells were labeled with [2-3H]mannose for 15-20 min and then chased with unlabeled mannose for various times up to 5 h. Lysosomal .beta.-glucuronidase was immunopptd. and its oligosaccharide units examd. for extent of phosphorylation and uncovering. The 1st phosphorylated oligosaccharides were detected after 20 min of labeling. Most of the phosphorylation occurred during the 1st 40 min of the chase period, and a max. of 30% of the oligosaccharide units were eventually phosphorylated. Oligosaccharides with 1 and 2 phosphodiesters were found. The earliest detectable phosphorylated species were devoid of the glucose residues known to be present on the lipid-linked oligosaccharide precursor. Uncovering of the phosphodiesters began shortly after the oligosaccharides were phosphorylated and occurred concomitantly with the removal of outer mannose residues. Taken together, these data demonstrate that phosphorylation of lysosomal enzyme oligosaccharides is a post-translational event. Proteolytic fragmentation of [3H]mannose-labeled .beta.-glucuronidase and partial digestion of [3H]leucine-labeled .beta.-glucuronidase with endo-.beta.-N-acetylglucosaminidase H suggest that there are 3 glycosylation sites per subunit. Each glycosylation site is partially phosphorylated. A portion of the high-mannose oligosaccharides at 1 site are processed to complex-type units.

L34 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

1981:437849 Document No. 95:37849 UDP-N-acetylglucosamine:glycoprotein N-acetylglucosamine-1-phosphotransferase. Proposed enzyme for the phosphorylation of the high mannose oligosaccharide units of lysosomal enzymes. Reitman, Marc L.; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Journal of Biological Chemistry, 256(9), 4275-81 (English) 1981. CODEN: JBCHA3. ISSN: 0021-9258.

AB The recognition marker for the targeting of lysosomal enzymes contains mannose 6-phosphate. The recent discovery of phosphate in diester linkage between N-acetylglucosamine (GlcNAc) and mannose in newly synthesized .beta.-glucuronidase led to the proposal that the phosphate might be acquired via N-acetylglucosamine phosphate transfer from UDP-GlcNAc. The synthesis of [.beta.-32P]UDP-[3H]GlcNAc and the use of this compd. to demonstrate a UDP-acetylglucosamine-glycoprotein acetylglucosamine-1-phosphotransferase is described. The basis of the enzyme assay is the incorporation of 32P and 3H into glycoproteins with a high affinity for Concanavalin A-Sepharose. This membrane-assocd. transferase is neither inhibited by tunicamycin nor stimulated by dolichol phosphate, indicating that the reaction does not proceed via a dolichylpyrophosphoryl-N-acetylglucosamine intermediate. Characterization of the enzyme reaction products (derived from either endogenous or exogenous acceptors) demonstrated that .alpha.-linked ***N*** - ***acetylglucosamine*** ***1*** - ***phosphate*** is transferred en bloc to the 6-hydroxyl of mannose in high-mannose oligosaccharides of glycoproteins. It is proposed that the function of this enzyme is to donate ***N*** - ***acetylglucosamine*** ***1*** - ***phosphate*** to mannose residues of newly synthesized lysosomal enzymes.

L34 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN
1981:206670 Document No. 94:206670 Fibroblasts from patients with I-cell disease and pseudo-Hurler polydystrophy are deficient in uridine 5'-diphosphate-N-acetylglucosamine: glycoprotein N-acetylglucosaminylphosphotransferase activity. Reitman, Marc L.; Varki, Ajit; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Journal of Clinical Investigation, 67(5), 1574-9 (English) 1981. CODEN: JCINAO. ISSN: 0021-9738.
AB Fibroblasts from patients with the lysosomal enzyme storage diseases, mucolipidosis II and III were severely deficient in UDP-N-acetylglucosamine:glycoprotein N-acetylglucosaminylphosphotransferase (I), the first enzyme in the sequence of tagging newly formed acid hydrolases for transport to lysosomes. The I activity (assayed using endogenous acceptors) in cultures from 6 normal subjects was 0.67-1.46 pmol ***N*** - ***acetylglucosamine*** - ***1*** - ***phosphate*** transferred/mg protein/h, whereas fibroblasts from 5 patients with mucolipidosis II and 5 patients with mucolipidosis III transferred <0.02 pmol/mg protein/h. The activity in 5 other mucolipidosis III cultures ranged from 0.02-0.27 pmol transferred/mg protein/h. The activity of .alpha.-N-acetylglucosaminylphosphodiesterase, the enzyme responsible for phosphomonoester exposure, is normal or elevated in cultured fibroblasts from both groups of the patients. The deficiency of I explains the biochem. abnormalities previously obsd. in mucolipidosis II and III.

L34 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN
1965:76678 Document No. 62:76678 Original Reference No. 62:13612g-h,13613a Thymidine 5'-diphosphate N-acetyl-D-glucosamine pyrophosphorylase activity of hog gastric mucosa. Kornfeld, Rosalind; ***Kornfeld, Stuart*** ; Ginsburg, Victor (Natl. Insts. of Health, Bethesda, MD). Biochemical and Biophysical Research Communications, 17(5), 578-81 (English) 1964. CODEN: BBRCA9. ISSN: 0006-291X.
AB Hog gastric mucosa exts. were tested for their ability to form sugar nucleotide precursors of mammalian heterosaccharides from N-acetyl-D-glucosamine 1-phosphate and various nucleotide triphosphates. These exts. could form dTDP N-acetyl-D-glucosamine (I) and epimerize this compd. to dTDP N-acetyl-D-galactosamine in the presence of DPN. However, expts. in vivo cast doubt on the physiol. importance of these reactions. After a large scale run, I was isolated by chromatography and had the chromatographic mobilities and spectrum characteristic of authentic I. A 50-lb. hog was injected with glucosamine-14C and after 13 min. the stomach was removed and its mucosa extd. with boiling 70% EtOH. Unlabeled carrier I was added to the ext. and both I and UDP N-acetyl-D-glucosamine were isolated by paper chromatography. Radioactivity of the sugar nucleotides on assay showed the original ext. had 60,000 count/min. as UDP N-acetylhexosamine and <50 counts/min. as dTDP N-acetylhexosamine. This suggests that, although hog gastric mucosa can make dTDP N-acetylhexosamine from N-acetyl-D-glucosamine 1-phosphate and dTTP, a deoxythymidine-linked pathway of hexosamine metabolism does not operate in vivo.

=> S L30 AND L23
L35 21 L30 AND L23

=> S L35 NOT (L10,L12,L20,L34)
L36 14 L35 NOT ((L10 OR L12 OR L20 OR L34))

=> D 1-14 CBIB ABS

L36 ANSWER 1 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN
2000:178341 Document No. 132:306803 Molecular basis of variant pseudo-Hurler polydystrophy (mucolipidosis IIIC). Raas-Rothschild, Annick; Cormier-Daire, Valerie; Bao, Ming; Genin, Emmanuelle; Salomon, Remi; Brewer, Kevin; Zeigler, Marsha; Mandel, Hanna; Toth, Steve; Roe, Bruce; Munnich, Arnold; ***Canfield, William M.*** (Department of Human Genetics, Hadassah Hebrew University Hospital, Jerusalem, 91120, Israel). Journal of Clinical Investigation, 105(5), 673-681 (English) 2000. CODEN: JCINAO. ISSN: 0021-9738. Publisher: American Society for Clinical Investigation.
AB Mucolipidosis IIIC (MLIIIC), or variant pseudo-Hurler polydystrophy, is an autosomal recessive disease of ***lysosomal*** ***hydrolase*** trafficking. Unlike the related diseases, mucolipidosis II and IIIA, the enzyme affected in mucolipidosis IIIC (N-Acetylglucosamine-1-phosphotransferase [GlcNAc-phosphotransferase]) retains full transferase activity on synthetic substrates but lacks activity on ***lysosomal*** ***hydrolases*** . Bovine GlcNAc-phosphotransferase has recently been isolated as a multisubunit enzyme with the subunit structure .alpha.2.beta.2.gamma.2. We cloned the cDNA for the human .gamma.-subunit and localized its gene to chromosome 16p. We also showed, in a large multiplex Druze family that exhibits this disorder, that MLIIIC also maps to this chromosomal region. Sequence anal. of the .gamma.-subunit cDNA in patients from 3 families identified a frameshift mutation, in codon 167 of the .gamma. subunit, that segregated with the disease, indicating MLIIIC results from mutations in the phosphotransferase .gamma.-subunit gene. This is to our knowledge the first description of the mol. basis for a human mucolipidosis and suggests that the .gamma. subunit functions in ***lysosomal*** ***hydrolase*** recognition.

L36 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN
1998:592720 Document No. 129:299526 Purification and multimeric structure of bovine N-acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase. Kornfeld, Rosalind; Bao, Ming; Brewer, Kevin; Noll, Carolyn; ***Canfield, William M.*** (Department of Medicine, Washington University School of Medicine, St. Louis, MO, 63110, USA). Journal of Biological Chemistry, 273(36), 23203-23210 (English) 1998. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB N-Acetylglucosamine-1-phosphodiester .alpha.-N-acetylglucosaminidase (EC 3.1.4.45; phosphodiester .alpha.-GlcNAcase) catalyzes the second step in the synthesis of the mannose 6-phosphate determinant required for efficient intracellular targeting of newly synthesized ***lysosomal*** ***hydrolases*** to the ***lysosome*** . A partially purified prepn. of phosphodiester .alpha.-GlcNAcase from bovine pancreas was used to generate a panel of murine monoclonal antibodies. The anti-phosphodiester .alpha.-GlcNAcase monoclonal antibody UC1 was coupled to a solid support and used to immunopurify the bovine liver enzyme 670,000-fold in 2 steps to apparent homogeneity with an overall yield of 14%. The purified phosphodiester .alpha.-GlcNAcase has a specific activity of 498 .mu.mol of [3H]GlcNAc-.alpha.-phosphomannose-.alpha.-Me cleaved per h per mg of protein using 0.5 mM [3H]GlcNAc-.alpha.-phosphomannose-.alpha.-Me as substrate. The subunit structure of the enzyme was detd. using a combination of anal. gel filtration chromatog., SDS-PAGE, and N-terminal sequencing. The data indicate that bovine phosphodiester .alpha.-GlcNAcase is a 272,000-Da complex of 4 identical 68,000-Da glycoprotein subunits arranged as 2 disulfide-linked homodimers. A sol. form of the enzyme, isolated from fetal bovine serum, showed the same subunit structure. Both forms of the enzyme reacted with a rabbit antibody raised to the N-terminal peptide of the liver enzyme, suggesting that phosphodiester .alpha.-GlcNAcase is a type I membrane-spanning glycoprotein with its N-terminus in the lumen of the Golgi app.

L36 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1997:510474 Document No. 127:118899 The phosphorylation of bovine DNase I Asn-linked oligosaccharides is dependent on specific lysine and arginine residues. Nishikawa, Atsushi; Gregory, Walter; Frenz, John; Cacia, Jerry; ***Kornfeld, Stuart*** (Department Medicine, Washington University School Medicine, St. Louis, MO, 63110, USA). Journal of Biological Chemistry, 272(31), 19408-19412 (English) 1997. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB The secretory glycoprotein, DNase I (I), acquires mannose 6-phosphate moieties on its Asn-linked oligosaccharides, indicating that it is a substrate for lysosomal enzyme N-acetylglucosamine-1-phosphotransferase (II). II recognizes a conformation-dependent protein determinant that is present in ***lysosomal*** ***hydrolases*** , but absent in most secretory glycoproteins. To identify the amino acid residues of I that are required for interaction with II, wild-type and mutant forms of bovine I were expressed in COS-1 cells and the extent of oligosaccharide phosphorylation detd. Phosphorylation of I oligosaccharides decreased from 12.6 to 2.3% when Lys-50, Lys-124, and Arg-27 were mutated to Ala, indicating that these residues are required for the basal level of phosphorylation. Mutation of Lys at other positions did not impair phosphorylation, demonstrating the selectivity of this process. When Arg-27 was replaced with a Lys, phosphorylation increased to 54%, showing that II prefers Lys to Arg residues. Mutation of Asn-74 to Lys also increased phosphorylation to 50.3%, and the double mutant (R27K/N74K) was phosphorylated 79%, equiv. to the values obtained with ***lysosomal*** ***hydrolases*** . Interestingly, Lys-27 and Lys-74 caused selective phosphorylation of the neighboring Asn-linked oligosaccharide. Finally, mutation of Lys-117 to Ala stimulated phosphorylation, demonstrating that some residues may be neg. regulators of this process. It was concluded that selected Lys and Arg residues on the surface of DNase I constitute the major elements in the II recognition domain present on this secretory glycoprotein.

L36 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1996:152281 Document No. 124:229294 The biogenesis of the MHC class II compartment in human I-cell disease B lymphoblasts. Glickman, Jonathan N.; Morton, Phillip A.; Slot, Jan W.; ***Kornfeld, Stuart*** ; Geuze, Hans J. (Dep. Medicine, Washington Univ. School Medicine, St. Louis, MO, 63110, USA). Journal of Cell Biology, 132(5), 769-85 (English) 1996. CODEN: JCLBA3. ISSN: 0021-9525. Publisher: Rockefeller University Press.

AB The localization and intracellular transport of major histocompatibility complex (MHC) class II mols. and ***lysosomal*** ***hydrolases*** were studied in I-Cell Disease (ICD) B lymphoblasts, which possess a mannose 6-phosphate (Man-6-P)-independent targeting pathway for lysosomal enzymes. In the trans-Golgi network (TGN), MHC class II-invariant chain complexes colocalized with the ***lysosomal*** ***hydrolase*** cathepsin D in buds and vesicles that lacked markers of clathrin-coated vesicle-mediated transport. These vesicles fused with the endocytic pathway leading to the formation of "early" MHC class II-rich compartments (MIICs). Similar structures were obsd. in the TGN of normal .beta. lymphoblasts although they were less abundant. Metabolic labeling and subcellular fractionation expts. indicated that newly synthesized cathepsin D and MHC class II-invariant chain complexes enter a non-clathrin-coated vesicular structure after their passage through the TGN and segregation from the secretory pathway. These vesicles were also devoid of the cation-dependent mannose 6-phosphate (Man-6-P) receptor, a marker of early and late endosomes. These findings suggest that in ICD B lymphoblasts the majority of MHC class II mols. are transported directly from the TGN to "early" MIICs and that acid hydrolases can be incorporated into MIICs simultaneously by a Man-6-P-independent process.

L36 ANSWER 5 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1992:587145 Document No. 117:187145 Lysosomal enzyme phosphorylation. I. Protein recognition determinants in both lobes of procathepsin D mediate its interaction with UDP-GlcNAc:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase. Baranski, Thomas J.; Cantor, Alan B.; ***Kornfeld,*** *** Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Journal of Biological Chemistry, 267(32), 23342-8 (English) 1992. CODEN: JBCHA3. ISSN: 0021-9258.

AB The nature of a protein domain that is shared among ***lysosomal*** ***hydrolases*** and is recognized by the UDP-GlcNAc:lysosomal enzyme

N-acetyl-glucosamine-1-phosphotransferase, the initial enzyme in the biosynthesis of mannose 6-phosphate residues, was investigated. Previously, elements of this recognition domain were identified using a chimeric protein approach. The combined substitution of two regions (amino acids 188-230, particularly lysine 203, and 265-292) from the carboxyl lobe of the ***lysosomal*** ***hydrolase*** cathepsin D into the homologous positions of the related secretory protein glycopepsinogen was sufficient to confer recognition by phosphotransferase and subsequent phosphorylation of the oligosaccharides when this chimeric protein was expressed in Xenopus oocytes. (Baranski, T. J., et al., 1990). The current study demonstrates that when these two regions are replaced in cathepsin D by the homologous glycopepsinogen amino acids, the resultant chimeric mol. is poorly phosphorylated. However, when either of these regions is substituted individually, the chimeric mols. are well phosphorylated. The phosphorylation of these latter chimeric proteins is dependent on the presence of procathepsin D amino lobe elements. By analyzing a series of chimeric proteins that contain all eight combinations of three consecutive segments of the entire amino lobe of procathepsin D, it was found that multiple regions of the amino lobe of cathepsin D enhance phosphorylation of the chimeric proteins. These elements may be part of an extended carboxyl lobe recognition domain or comprise a second independent recognition domain.

L36 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1991:627034 Document No. 115:227034 Mapping and molecular modeling of a recognition domain for lysosomal enzyme targeting. Baranski, Thomas J.; Koelsch, Gerald; Hartsuck, Jean A.; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Journal of Biological Chemistry, 266(34), 23365-72 (English) 1991. CODEN: JBCHA3. ISSN: 0021-9258.

AB Lysosomal enzymes contain a common protein determinant that is recognized by UDP-GlcNAc:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase, the initial enzyme in the biosynthesis of mannose-6-phosphate residues. Previously, a lysosomal enzyme recognition domain was generated by substituting two regions (lysine 203 and amino acids 265-292) of the ***lysosomal*** ***hydrolase*** cathepsin D into a related secretory protein glycopepsinogen. When expressed in Xenopus oocytes, the oligosaccharides of the chimeric protein were efficiently phosphorylated (Baranski, T. J. et al., 1990). In the current study, incremental substitutions of cathepsin D residues into glycopepsinogen and alanine-scanning mutagenesis were utilized to define the recognition domain more precisely. A computer-generated model of the cathepsin D/pepsinogen chimeric mol. served as a guide for mutagenesis and for the interpretation of results. These studies indicate that the recognition domain is a surface patch that contains multiple interacting sites. There is a strict positional requirement for the lysine residue at position 203.

L36 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1990:627089 Document No. 113:227089 Generation of a lysosomal enzyme targeting signal in the secretory protein pepsinogen. Baranski, Thomas J.; Faust, Phyllis L.; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Cell (Cambridge, MA, United States), 63(2), 281-91 (English) 1990. CODEN: CELLB5. ISSN: 0092-8674.

AB Lysosomal enzymes contain a common protein determinant that is recognized by lysosomal enzyme N-acetylglucosamine-1-phosphotransferase, the initial enzyme in the formation of mannose 6-phosphate residues. To identify this protein determinant, chimeric mols. between 2 aspartyl proteases, cathepsin D, a lysosomal enzyme, and pepsinogen, a secretory protein, were constructed. When expressed in Xenopus oocytes, the oligosaccharides of cathepsin D were efficiently phosphorylated, whereas the oligosaccharides of a glycosylated form of pepsinogen were not phosphorylated. The combined substitution of 2 noncontinuous sequences of cathepsin D (lysine-203 and amino acids 265-292) into the analogous positions of glycopepsinogen resulted in phosphorylation of the oligosaccharides of the expressed chimeric mol. These 2 sequences were in direct apposition on the surface of the mol., indicating that amino acids from different regions come together in 3-dimensional space to form this recognition domain. Other regions of cathepsin D were identified that may be components of a more extensive recognition marker.

L36 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1985:467440 Document No. 103:67440 Lysosomal enzyme binding to mouse P388D1 macrophage membranes lacking the 215-kDa mannose 6-phosphate receptor: Evidence for the existence of a second mannose 6-phosphate receptor. Hoflack, Bernard; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Proceedings of the National Academy of Sciences of the United States of America, 82(13), 4428-32 (English) 1985. CODEN: PNASA6. ISSN: 0027-8424.

AB Mouse P388D1 macrophages target newly synthesized acid ***hydrolases*** to ***lysosomes*** in spite of their lack of the 215-kilodalton (kDa) mannose 6-phosphate (Man-6-P) receptor. These cells contain a membrane-assocd. Man-6-P receptor that is distinct from the previously described receptor. The new receptor binds lysosomal enzymes contg. phosphomannosyl residues. This binding is inhibited by Man-6-P or by pretreatment of the lysosomal enzymes with alk. phosphatase. Lysosomal enzyme binding occurs at neutral pH and dissocn. of the bound ligand occurs at low pH values comparable to those found within endosomes or lysosomes. The new receptor differs from the 215-kDa Man-6-P receptor in 2 ways. It has an abs. requirement for divalent cations and is unable to bind Dictyostelium discoideum lysosomal enzymes, which contain methylphosphomannosyl residues rather than the usual phosphomannosyl monoesters. Based on the difference in cation requirement, the 215-kDa receptor may be referred to as Man-6-P receptor CI (cation independent) and the new receptor as Man-6-P receptor CD (cation dependent). The Man-6-P receptor CD apparently functions in the targeting of newly synthesized acid ***hydrolases*** to ***lysosomes*** in P388D1 macrophages.

L36 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1985:201574 Document No. 102:201574 Evidence for a mannose 6-phosphate-independent pathway for lysosomal enzyme targeting. Gabel, Christopher A.; Goldberg, Daniel E.; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, USA). Mol. Basis Lysosomal Storage Disord., [Proc. Conf.], Meeting Date 1983, 175-93. Editor(s): Barranger, John A.; Brady, Roscoe O. Academic: Orlando, Fla. (English) 1984. CODEN: 53OHA7.

AB A transport system, independent of mannose 6-phosphate (M-6-P) receptors, for targeting ***lysosomal*** ***hydrolases*** in human and other animal cells is discussed. [125I].beta.-hexosaminidase was efficiently imported into cultured cells deficient in M-6-P receptors; this process was not inhibited by M-6-P. Lysosomal enzymes in receptor-deficient cells are phosphorylated in their oligosaccharide moieties as in normal cells, but are not rapidly dephosphorylated after uptake by lysosomes, in contrast to normal cells. The implication of these findings are discussed.

L36 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1984:469952 Document No. 101:69952 Targeting of .beta.-glucuronidase to lysosomes in mannose 6-phosphate receptor-deficient MOPC 315 cells. Gabel, Christopher A.; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Journal of Cell Biology, 99(1, Pt. 1), 296-305 (English) 1984. CODEN: JCLBA3. ISSN: 0021-9525.

AB The murine plasma cell line MOPC 315 efficiently targets newly synthesized acid ***hydrolases*** to ***lysosomes*** in spite of a marked deficiency in the level of the mannose 6-phosphate receptor. To better understand the routing of lysosomal enzymes in this cell line, pulse-chase expts. were performed with [2-3H]mannose and [35S]methionine followed by immunopptn. of .beta.-glucuronidase and IgA. By 3 h of chase, essential all of the newly synthesized .beta.-glucuronidase had undergone proteolytic processing, suggesting that the mols. had reached lysosomes. At this time 30% of the pulse-labeled IgA was still intracellular. The oligosaccharides on the intracellular IgA were of the high mannose-type, whereas the secreted IgA contained processed, complex-type oligosaccharides. Thus, the intracellular IgA was still in the endoplasmic reticulum or an early region of the Golgi complex when all of the .beta.-glucuronidase had reached lysosomes. Therefore, .beta.-glucuronidase and IgA must exit from the endoplasmic reticulum or the early Golgi complex at different rates, a finding that is inconsistent with bulk phase movement of these proteins from the endoplasmic reticulum to the trans Golgi complex. The addn. of the ionophore monensin greatly slows the rate of IgA secretion from MOPC 315 cells and the mols. secreted have incompletely processed oligosaccharides. In contrast, monensin only

slightly delays the transport of newly synthesized .beta.-glucuronidase to lysosomes and caused no significant alteration in the extent of oligosaccharide phosphorylation, a process that appears to occur in the early cis Golgi complex. However, the labeled .beta.-glucuronidase was deficient in sialylated, phosphorylated hybrid oligosaccharides whose biosynthesis requires the action of late stage oligosaccharide processing enzymes assumed to be localized in the trans Golgi complex.

L36 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN
1983:608782 Document No. 99:208782 Structural analysis of the asparagine-linked oligosaccharides from three lysosomal enzymes of Dictyostelium discoideum. Evidence for an unusual acid-stable phosphodiester. Freeze, Hudson H.; Yeh, Richard; Miller, Arnold L.; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Journal of Biological Chemistry, 258(24), 14874-9 (English) 1983. CODEN: JBCHA3. ISSN: 0021-9258.

AB Lysosomal enzymes of D. discoideum contain mannose 6-phosphate and bind with high affinity to the phosphomannosyl receptor of human fibroblasts. In this study, the asparagine-linked oligosaccharide units present on these enzymes were partially characterized. [3H]mannose-labeled .alpha.-mannosidase, .beta.-glucosidase, and .beta.-N-acetylglucosaminidase were purified from the spent growth medium of strain AX3 and glycopeptides were prepd. by pronase digestion. Approx. 75% of the glycopeptides contained sulfate residues. These could be removed by solvolysis without degrading the underlying oligosaccharide. Following solvolysis (but not before), the oligosaccharides could be released by endo-.beta.-N-acetylglucosaminidase H, indicating the presence of high mannose-type units. Greater than 85% of the oligosaccharides contained 1 or 2 mannose 6-phosphate residues in the form of an unusual acid-stable phosphodiester. About 3% of the oligosaccharides contained phosphomonoesters and only 6% were neutral species. The major neutral oligosaccharide eluted in the position of Man9GlcNAc when analyzed by high-performance liq. chromatog., whereas the minor species appeared to be 1-2 residues larger. Acetolysis of the major phosphorylated fractions revealed that mols. with a single mannose 6-phosphate contained the phosphomannosyl residue on the branch linked .alpha.1,6 to the .beta.-linked mannose, whereas mols. with 2 phosphomannosyl residues had the residues on this branch as well as the branch linked .alpha.1,3 to the .beta.-linked mannose. The mechanism of mannose phosphorylation in the slime mold must differ from that of mammalian cells since the phosphomannosyl residues are present as acid-resistant phosphodiesters rather than acid-labile phosphodiesters.

L36 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN
1983:86803 Document No. 98:86803 Identification and characterization of cells deficient in the mannose 6-phosphate receptor: evidence for an alternate pathway for lysosomal enzyme targeting. Gabel, Christopher A.; Goldberg, Daniel E.; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Proceedings of the National Academy of Sciences of the United States of America, 80(3), 775-9 (English) 1983. CODEN: PNASA6. ISSN: 0027-8424.

AB Newly synthesized lysosomal enzymes are known to acquire phosphomannosyl units, which allow binding of the enzymes to the mannose 6-phosphate receptor and subsequent translocation to lysosomes. In some cell types, this sequence of events is necessary for the delivery of these enzymes to lysosomes. With a slime mold ***lysosomal*** ***hydrolase*** as a probe, 3 murine cell lines that lack the receptor and 1 line that contains very low (3%) receptor activity were identified. Each of these lines synthesizes the mannose 6-phosphate recognition marker on its lysosomal enzymes, but, unlike cell lines with high levels of receptor, the cells accumulate oligosaccharides contg. phosphomonoesters. The receptor-deficient lines possess high levels of intracellular acid hydrolase activity, which is contained in dense granules characteristic of lysosomes. Apparently, intracellular mechanisms independent of the mannose 6-phosphate receptor must exist in some cells for the delivery of acid ***hydrolases*** to ***lysosomal*** organelles.

L36 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN
1983:67540 Document No. 98:67540 Steps in the phosphorylation of the high mannose oligosaccharides of lysosomal enzymes. ***Kornfeld, Stuart*** ; Reitman, Marc L.; Varki, Ajit; Goldberg, Daniel; Gabel, Christopher A.

(Sch. Med., Washington Univ., St Louis, MO, 63110, USA). Ciba Foundation Symposium, 92(Membr. Recycl.), 138-56 (English) 1982. CODEN: CIBSB4. ISSN: 0300-5208.

AB A review with 35 refs. on the phosphorylation path of ***lysosomal*** acid ***hydrolases*** by N-acetylglucosamine 1-phosphotransferase and .alpha.-N-acetylglucosamine phosphodiesterase.

L36 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1982:542296 Document No. 97:142296 Lysosomal enzyme phosphorylation in mouse lymphoma cell lines with altered asparagine-linked oligosaccharides. Gabel, Christopher A.; ***Kornfeld, Stuart*** (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). Journal of Biological Chemistry, 257(18), 10605-12 (English) 1982. CODEN: JBCHA3. ISSN: 0021-9258.

AB The targeting of acid ***hydrolases*** to ***lysosomes*** involves phosphorylation of mannose residues on these enzymes. To det. whether alterations in the structure of the oligosaccharide acceptor affect formation of the mannose 6-phosphate recognition marker, 2 mouse lymphoma cell lines altered in the assembly of asparagine-linked oligosaccharides were studied. Cells were labeled with [2-3H]mannose and the phosphorylated oligosaccharides were isolated and characterized. PhaR2.7 cells, which are deficient in glucosidase II activity, formed less of oligosaccharide-bound mannose 6-phosphate than parent cells. The major phosphorylated oligosaccharide formed by PhaR2.7 contained a single phosphodiester located on the branch linked .alpha.-(1.fwdarw.6) to the .beta.-linked mannose; the majority of these mols. were glucosylated. Relatively few oligosaccharides contg. 2 phosphate esters were isolated and those formed were not glucosylated. This suggests that glucose residues present on high-mannose oligosaccharides prevent phosphorylation of mannose residues on the branch linked .alpha.-(1.fwdarw.3) to the .beta.-linked mannose. In contrast, Thy-1- cells synthesized a normal level of oligosaccharide-bound mannose 6-phosphate despite forming truncated, high-mannose-type units. These oligosaccharides contained only 4 mannose residues and a single phosphate, present as either a phosphodiester or phosphomonoester. .beta.-Galactosidase isolated from Thy-1- cells bound to human fibroblast membranes and was endocytosed by intact fibroblasts to a similar extent as parent enzyme. Thus, altered high-mannose-type oligosaccharides on lysosomal enzymes are phosphorylated. However, the extent of phosphorylation and the distribution of the esters is dependent upon the structure of the acceptor oligosaccharide. In addn., the presence of monophosphorylated oligosaccharides on acid hydrolases is sufficient for their recognition and translocation by the mannose 6-phosphate receptor.

	L #	Hits	Search Text	DBs
1	L1	74	n adj acetylglucosamine adj "1" adj phosphate	USPAT ; US-PG PUB
2	L2	12677	oligosaccharide	USPAT ; US-PG PUB
3	L3	27827	pseudomonas	USPAT ; US-PG PUB
4	L4	3218	exotoxin adj A	USPAT ; US-PG PUB
5	L5	1	exodotoxin adj A	USPAT ; US-PG PUB
6	L6	960	L3 ADJ L4	USPAT ; US-PG PUB
7	L7	1	L3 ADJ L5	USPAT ; US-PG PUB
8	L8	6221	LYSOSOM$	USPAT ; US-PG PUB
9	L9	7535	HYDROLASE	USPAT ; US-PG PUB
10	L10	224	L8 ADJ L9	USPAT ; US-PG PUB
11	L11	10	L10 AND L4	USPAT ; US-PG PUB
12	L12	1	L1 AND L4	USPAT ; US-PG PUB
13	L13	322	L2 AND L4	USPAT ; US-PG PUB
14	L15	1	L14 AND L1	USPAT ; US-PG PUB
15	L16	0	L2 NEAR8 L6	USPAT ; US-PG PUB

	L #	Hits	Search Text	DBs
16	L17	1	L2 SAME L6	USPAT ; US-PG PUB
17	L18	0	L17 NOT L15	USPAT ; US-PG PUB
18	L14	77	L2 AND L6	USPAT ; US-PG PUB

Title: US-10-023-894-17

RESULT 2
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VERSION AF187072.1 GI:6425039
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ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 2183)
AUTHORS Kornfeld,R., Bao,M., Brewer,K., Noll,C. and Canfield,W.
TITLE Molecular cloning and functional expression of two splice forms of human N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase
JOURNAL J. Biol. Chem. 274 (46), 32778-32785 (1999)
MEDLINE 20020246
PUBMED 10551838
REFERENCE 2 (bases 1 to 2183)
AUTHORS Kornfeld,R., Bao,M., Brewer,K., Noll,C., Pan,H., Roe,B. and Canfield,W.M.
TITLE Direct Submission
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ORIGIN

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Qy        961 GTGGTGTGTGTGCACGAACCCCGCTGCCAGCCGCCTGACTGCCACGGCCACGGGACCTGC 1020
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Db        961 GTGGTGTGTGTGCACGAACCCCGCTGCCAGCCGCCTGACTGCCACGGCCACGGGACCTGC 1020

Qy       1021 GTGGACGGGCACTGCCAATGCACCGGGCACTTCTGGCGGGGTCCCGGCTGTGATGAGCTG 1080
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1021 GTGGACGGGCACTGCCAATGCACCGGGCACTTCTGGCGGGGTCCCGGCTGTGATGAGCTG 1080

Qy       1081 GACTGTGGCCCCTCTAACTGCAGCCAGCACGGACTGTGCACGGAGACCGGCTGCCGCTGT 1140
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1081 GACTGTGGCCCCTCTAACTGCAGCCAGCACGGACTGTGCACGGAGACCGGCTGCCGCTGT 1140

Qy       1141 GATGCCGGATGGACCGGGTCCAACTGCAGTGAAGAGTGTCCCCTTGGCTGGCATGGGCCG 1200
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1141 GATGCCGGATGGACCGGGTCCAACTGCAGTGAAGAGTGTCCCCTTGGCTGGCATGGGCCG 1200

Qy       1201 GGCTGCCAGAGGCGTTGTAAGTGTGAGCACCATTGTCCCTGTGACCCCAAGACTGGCAAC 1260
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1201 GGCTGCCAGAGGCGTTGTAAGTGTGAGCACCATTGTCCCTGTGACCCCAAGACTGGCAAC 1260
```

```
Qy       1261 TGCAGCGTCTCCAGAGTAAAGCAGTGTCTCCAGCCACCTGAAGCCACCCTGAGGGCGGGA 1320
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1261 TGCAGCGTCTCCAGAGTAAAGCAGTGTCTCCAGCCACCTGAAGCCACCCTGAGGGCGGGA 1320

Qy       1321 GAACTCTCCTTTTTCACCAGGACCGCCTGGCTAGCCCTCACCCTGGCGCTGGCCTTCCTC 1380
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1321 GAACTCTCCTTTTTCACCAGGACCGCCTGGCTAGCCCTCACCCTGGCGCTGGCCTTCCTC 1380

Qy       1381 CTGCTGATCAGCATTGCAGCAAACCTGTCCTTGCTCCTGTCCAGAGCAGAGAGGAACCGG 1440
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1381 CTGCTGATCAGCATTGCAGCAAACCTGTCCTTGCTCCTGTCCAGAGCAGAGAGGAACCGG 1440

Qy       1441 CGCCTGCATGGGGACTATGCATACCACCCGCTGCAGGAGATGAACGGGGAGCCTCTGGCC 1500
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1441 CGCCTGCATGGGGACTATGCATACCACCCGCTGCAGGAGATGAACGGGGAGCCTCTGGCC 1500

Qy       1501 GCAGAGAAGGAGCAGCCAGGGGGCGCCCACAACCCCTTCAAGGACTGAAGCCTCAAGCTG 1560
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Qy       1561 CCCGGGGTGGCACGTCGCGAAAGCTTGTTTCCCCACGGTCTGGCTTCTGCAGGGGAAATT 1620
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Db       1561 CCCGGGGTGGCACGTCGCGAAAGCTTGTTTCCCCACGGTCTGGCTTCTGCAGGGGAAATT 1620

Qy       1621 TCAAGGCCACTGGCGTGGACCATCTGGGTGTCCTCAATGGCCCCTGTGGGGCAGCCAAGT 1680
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1621 TCAAGGCCACTGGCGTGGACCATCTGGGTGTCCTCAATGGCCCCTGTGGGGCAGCCAAGT 1680

Qy       1681 TCCTGATAGCACTTGTGCCTCAGCCCCTCACCTGGCCACCTGCCAGGGCACCTGCAACCC 1740
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Db       1681 TCCTGATAGCACTTGTGCCTCAGCCCCTCACCTGGCCACCTGCCAGGGCACCTGCAACCC 1740

Qy       1741 TAGCAATACCATGCTCGCTGGAGAGGCTCAGCTGCCTGCTTCTCGCCTGCCTGTGTCTGC 1800
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Db       1741 TAGCAATACCATGCTCGCTGGAGAGGCTCAGCTGCCTGCTTCTCGCCTGCCTGTGTCTGC 1800

Qy       1801 TGCCGAGAAGCCCGTGCCCCCGGGAGGGCTGCCGCACTGCCAAAGAGTCTCCCTCCTCCT 1860
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Qy       1861 GGGGAAGGGGCTGCCAACGAACCAGACTCAGTGACCACGTCATGACAGAACAGCACATCC 1920
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Db       1861 GGGGAAGGGGCTGCCAACGAACCAGACTCAGTGACCACGTCATGACAGAACAGCACATCC 1920

Qy       1921 TGGCCAGCACCCCTGGCTGGAGTGGGTTAAAGGGACGAGTCTGCCTTCCTGGCTGTGACA 1980
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Db       1921 TGGCCAGCACCCCTGGCTGGAGTGGGTTAAAGGGACGAGTCTGCCTTCCTGGCTGTGACA 1980

Qy       1981 CGGGACCCCTTTTCTACAGACCTCATCACTGGATTTGCCAACTAGAATTCGATTTCCTGT 2040
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Db       1981 CGGGACCCCTTTTCTACAGACCTCATCACTGGATTTGCCAACTAGAATTCGATTTCCTGT 2040

Qy       2041 CATAGGAAGCTCCTTGGAAGAAGGGATGGGGGGATGAAATCATGTTTACAGACCTGTTTT 2100
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Db       2041 CATAGGAAGCTCCTTGGAAGAAGGGATGGGGGGATGAAATCATGTTTACAGACCTGTTTT 2100

Qy       2101 GTCATCCTGCTGCCAAGAAGTTTTTTAATCACTTGAATAAATTGATATAATAAAAGGAGC 2160
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       2101 GTCATCCTGCTGCCAAGAAGTTTTTTAATCACTTGAATAAATTGATATAATAAAAGGAGC 2160

Qy       2161 CACCAGGTGGTGTGTGGATTCTG 2183
             |||||||||||||||||||||||
Db       2161 CACCAGGTGGTGTGTGGATTCTG 2183
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Title: US-10-023-894-18

RESULT 1
US-09-635-872A-6
; Sequence 6, Application US/09635872A
; Patent No. 6534300
; GENERAL INFORMATION:
; APPLICANT: CANFIELD, WILLIAM
; TITLE OF INVENTION: METHODS FOR PRODUCING HIGHLY PHOSPHORYLATED LYSOSOMAL HYDROLASES
; FILE REFERENCE: 195613US0
; CURRENT APPLICATION NUMBER: US/09/635,872A
; CURRENT FILING DATE: 2000-08-10
; PRIOR APPLICATION NUMBER: 60/153,831
; PRIOR FILING DATE: 1999-09-14
; NUMBER OF SEQ ID NOS: 52
; SOFTWARE: PatentIn version 3.0
; SEQ ID NO 6
; LENGTH: 515
; TYPE: PRT
; ORGANISM: Homo sapiens
; FEATURE:
; NAME/KEY: SIGNAL
; LOCATION: (1)..(24)
; NAME/KEY: PROPEP
; LOCATION: (25)..(49)
US-09-635-872A-6

Query Match 100.0%; Score 2827; DB 4; Length 515;
Best Local Similarity 100.0%; Pred. No. 3.8e-237;
Matches 515; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

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Qy          1 MATSTGRWLLLRLALFGFLWEASGGLDSGASRDDDLLLPYPRARARLPRDCTRVRAGNRE 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MATSTGRWLLLRLALFGFLWEASGGLDSGASRDDDLLLPYPRARARLPRDCTRVRAGNRE 60

Qy         61 HESWPPPPATPGAGGLAVRTFVSHFRDRAVAGHLTRAVEPLRTFSVLEPGGPGGCAARRR 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 HESWPPPPATPGAGGLAVRTFVSHFRDRAVAGHLTRAVEPLRTFSVLEPGGPGGCAARRR 120

Qy        121 ATVEETARAADCRVAQNGGFFRMNSGECLGNVVSDERRVSSSGGLQNAQFGIRRDGTLVT 180
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Db        121 ATVEETARAADCRVAQNGGFFRMNSGECLGNVVSDERRVSSSGGLQNAQFGIRRDGTLVT 180

Qy        181 GYLSEEEVLDTENPFVQLLSGVVWLIRNGSIYINESQATECDETQETGSFSKFVNVISAR 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Qy        241 TAIGHDRKGQLVLFHADGHTEQRGINLWEMAEFLLKQDVVNAINLDGGGSATFVLNGTLA 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 TAIGHDRKGQLVLFHADGHTEQRGINLWEMAEFLLKQDVVNAINLDGGGSATFVLNGTLA 300

Qy        301 SYPSDHCQDNMWRCPRQVSTVVCVHEPRCQPPDCHGHGTCVDGHCQCTGHFWRGPGCDEL 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 SYPSDHCQDNMWRCPRQVSTVVCVHEPRCQPPDCHGHGTCVDGHCQCTGHFWRGPGCDEL 360

Qy        361 DCGPSNCSQHGLCTETGCRCDAGWTGSNCSEECPLGWHGPGCQRRCKCEHHCPCDPKTGN 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 DCGPSNCSQHGLCTETGCRCDAGWTGSNCSEECPLGWHGPGCQRRCKCEHHCPCDPKTGN 420

Qy        421 CSVSRVKQCLQPPEATLRAGELSFFTRTAWLALTLALAFLLLISIAANLSLLLSRAERNR 480
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Db        421 CSVSRVKQCLQPPEATLRAGELSFFTRTAWLALTLALAFLLLISIAANLSLLLSRAERNR 480

Qy        481 RLHGDYAYHPLQEMNGEPLAAEKEQPGGAHNPFKD 515
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Db        481 RLHGDYAYHPLQEMNGEPLAAEKEQPGGAHNPFKD 515
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RESULT 1
Q9UK23
ID Q9UK23 PRELIMINARY; PRT; 515 AA.

```
AC   Q9UK23;
DT   01-MAY-2000 (TrEMBLrel. 13, Created)
DT   01-MAY-2000 (TrEMBLrel. 13, Last sequence update)
DT   01-MAR-2003 (TrEMBLrel. 23, Last annotation update)
DE   N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase
DE   (EC 3.1.4.45).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=20020246; PubMed=10551838;
RA   Kornfeld R., Bao M., Brewer K., Noll C., Canfield W.M.;
RT   "Molecular Cloning and Functional Expression of Two Splice Forms of
RT   Human N-acetylglucosamine-1-phosphodiester alpha-N-
RT   acetylglucosaminidase.";
RL   J. Biol. Chem. 274:32778-32785(1999).
DR   EMBL; AF187072; AAF08273.1; -.
DR   HSSP; P05106; 1JV2.
DR   InterPro; IPR006209; EGF_like.
DR   InterPro; IPR006210; IEGF.
DR   SMART; SM00181; EGF; 1.
DR   PROSITE; PS00022; EGF_1; 1.
DR   PROSITE; PS01186; EGF_2; 1.
KW   EGF-like domain; Hydrolase.
SQ   SEQUENCE   515 AA;  56153 MW;  A56A6103C2D16809 CRC64;

  Query Match             100.0%;  Score 2827;  DB 4;  Length 515;
  Best Local Similarity   100.0%;  Pred. No. 2.4e-233;
  Matches  515;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 MATSTGRWLLLRLALFGFLWEASGGLDSGASRDDDLLLPYPRARARLPRDCTRVRAGNRE 60
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Db          1 MATSTGRWLLLRLALFGFLWEASGGLDSGASRDDDLLLPYPRARARLPRDCTRVRAGNRE 60

Qy         61 HESWPPPPATPGAGGLAVRTFVSHFRDRAVAGHLTRAVEPLRTFSVLEPGGPGGCAARRR 120
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Db         61 HESWPPPPATPGAGGLAVRTFVSHFRDRAVAGHLTRAVEPLRTFSVLEPGGPGGCAARRR 120

Qy        121 ATVEETARAADCRVAQNGGFFRMNSGECLGNVVSDERRVSSSGGLQNAQFGIRRDGTLVT 180
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Db        121 ATVEETARAADCRVAQNGGFFRMNSGECLGNVVSDERRVSSSGGLQNAQFGIRRDGTLVT 180

Qy        181 GYLSEEEVLDTENPFVQLLSGVVWLIRNGSIYINESQATECDETQETGSFSKFVNVISAR 240
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Db        181 GYLSEEEVLDTENPFVQLLSGVVWLIRNGSIYINESQATECDETQETGSFSKFVNVISAR 240

Qy        241 TAIGHDRKGQLVLFHADGHTEQRGINLWEMAEFLLKQDVVNAINLDGGGSATFVLNGTLA 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 TAIGHDRKGQLVLFHADGHTEQRGINLWEMAEFLLKQDVVNAINLDGGGSATFVLNGTLA 300

Qy        301 SYPSDHCQDNMWRCPRQVSTVVCVHEPRCQPPDCHGHGTCVDGHCQCTGHFWRGPGCDEL 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 SYPSDHCQDNMWRCPRQVSTVVCVHEPRCQPPDCHGHGTCVDGHCQCTGHFWRGPGCDEL 360

Qy        361 DCGPSNCSQHGLCTETGCRCDAGWTGSNCSEECPLGWHGPGCQRRCKCEHHCPCDPKTGN 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 DCGPSNCSQHGLCTETGCRCDAGWTGSNCSEECPLGWHGPGCQRRCKCEHHCPCDPKTGN 420

Qy        421 CSVSRVKQCLQPPEATLRAGELSFFTRTAWLALTLALAFLLLISIAANLSLLLSRAERNR 480
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Qy        481 RLHGDYAYHPLQEMNGEPLAAEKEQPGGAHNPFKD 515
              |||||||||||||||||||||||||||||||||||
Db        481 RLHGDYAYHPLQEMNGEPLAAEKEQPGGAHNPFKD 515
```

Search in ENZYME for: n-acetylglucosamine-1-phosphate

Release 33, October 2003, and updates up to 12-Oct-2003

Please choose one of the following entries:

2.7.7.23 UDP-N-acetylglucosamine diphosphorylase.
(AN: UDP-N-acetylglucosamine pyrophosphorylase.
N-acetylglucosamine-1-phosphate uridyltransferase.)

2.7.8.15 UDP-N-acetylglucosamine--dolichyl-phosphate
N-acetylglucosaminephosphotransferase.
(AN: N-acetylglucosamine-1-phosphate transferase.
GlcNAc-1-P transferase.)

NiceZyme View of ENZYME: EC 2.7.7.23

Official Name

UDP-N-acetylglucosamine diphosphorylase.

Alternative Name(s)

UDP-N-acetylglucosamine pyrophosphorylase.
N-acetylglucosamine-1-phosphate uridyltransferase.

Reaction catalysed

```
    UTP
  + N-acetyl-alpha-D-glucosamine 1-phosphate
<=>
    diphosphate
  + UDP-N-acetyl-D-glucosamine
```

Comments

- The enzyme from several bacteria has been shown to be bifunctional and also to possess the activity of EC 2.3.1.157.

Cross-references

Biochemical Pathways; map number(s)	D4, E4
PROSITE	PDOC00094
BRENDA	2.7.7.23
EMP/PUMA	2.7.7.23
WIT	2.7.7.23
Kyoto University LIGAND chemical database	2.7.7.23
IUBMB Enzyme Nomenclature	2.7.7.23
IntEnz	2.7.7.23
MEDLINE	Find literature relating to 2.7.7.23
Swiss-Prot	P42817, GCAD_BACCL; P28017, GCAD_BACME; P14192, GCAD_BACSU; P57139, GLMU_BUCAI; Q8KA74, GLMU_BUCAP; P17114, GLMU_ECOLI; P43889, GLMU_HAEIN; Q50986, GLMU_NEIGO; O64765, UAP1_ARATH; Q18493, UAP1_CAEEL; O74933, UAP1_CANAL; Q16222, UAP1_HUMAN; O94617, UAP1_SCHPO; P43123, UAP1_YEAST;

View entry in original ENZYME format
If you would like to retrieve all the Swiss-Prot entries referenced in this entry, click here.

NiceZyme View of ENZYME: EC 2.7.8.15

Official Name
UDP-N-acetylglucosamine--dolichyl-phosphate N-acetylglucosaminephosphotransferase.
Alternative Name(s)
N-acetylglucosamine-1-phosphate transferase. GlcNAc-1-P transferase.
Reaction catalysed
UDP-N-acetyl-D-glucosamine + dolichyl phosphate <=> UMP + N-acetyl-D-glucosaminyl-diphosphodolichol

Cross-references	
Biochemical Pathways; map number(s)	R5
BRENDA	2.7.8.15
EMP/PUMA	2.7.8.15
WIT	2.7.8.15
Kyoto University LIGAND chemical database	2.7.8.15
IUBMB Enzyme Nomenclature	2.7.8.15
IntEnz	2.7.8.15
MEDLINE	Find literature relating to 2.7.8.15
Swiss-Prot	P24140, GPT_CRIGR ; P23338, GPT_CRILO ; Q9H3H5, GPT_HUMAN ; P42864, GPT_LEIME ; P42867, GPT_MOUSE ; P42881, GPT_SCHPO ; P39465, GPT_SULAC ; P96000, GPT_SULSO ; P07286, GPT_YEAST ;

View entry in original ENZYME format

If you would like to retrieve all the Swiss-Prot entries referenced in this entry, click here.

Search in ENZYME for: N-acetylglucosaminidase

Release 33, October 2003, and updates up to 12-Oct-2003

Please choose one of the following entries:

3.1.4.45 N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase.
(AN: Alpha-N-acetylglucosaminyl phosphodiesterase.
Lysosomal alpha-N-acetylglucosaminidase.)

3.2.1.14 Chitinase.
(AN: Chitodextrinase.
1,4-beta-poly-N-acetylglucosaminidase.
Poly-beta-glucosaminidase.)

3.2.1.50 Alpha-N-acetylglucosaminidase.
(AN: N-acetyl-alpha-glucosaminidase.
NAG.)

3.2.1.96 Mannosyl-glycoprotein endo-beta-N-acetylglucosamidase.
(AN: Endo-beta-N-acetylglucosaminidase.
Di-N-acetylchitobiosyl beta-N-acetylglucosaminidase.)

3.2.2.11 Beta-aspartyl-N-acetylglucosaminidase.

NiceZyme View of ENZYME: EC 3.1.4.45

Official Name

N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase.

Alternative Name(s)

Alpha-N-acetylglucosaminyl phosphodiesterase.
Lysosomal alpha-N-acetylglucosaminidase.

Reaction catalysed

```
    Glycoprotein N-acetyl-D-glucosaminyl-phospho-D-mannose
  + H(2)O
<=>
    N-acetyl-D-glucosamine
  + glycoprotein phospho-D-mannose
```

Comments

- Acts on a variety of compounds in which N-acetyl-D-glucosamine is alpha-linked to a phosphate group, including the biosynthetic in intermediates of the high mannose oligosaccharide components of some lysosomal enzymes and the product of EC 2.7.8.17.

Cross-references

Biochemical Pathways; map number(s)	S3
BRENDA	3.1.4.45
EMP/PUMA	3.1.4.45
WIT	3.1.4.45
Kyoto University LIGAND chemical database	3.1.4.45
IUBMB Enzyme Nomenclature	3.1.4.45
IntEnz	3.1.4.45
MEDLINE	Find literature relating to 3.1.4.45

View entry in original ENZYME format

ENZYME - The Enzyme Data Bank

Search by description or alternative name

There is no ENZYME entry for **N-acetylglucosamine-1-phosphodiester-N-acetylglucosaminidase**. Please try again.

ENZYME - The Enzyme Data Bank

Search by description or alternative name

There is no ENZYME entry for **phosphodiester-alpha-GlcNAcase**. Please try again.

ENZYME - The Enzyme Data Bank

Search by description or alternative name

There is no ENZYME entry for **uncovering enzyme**. Please try again.